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Article in *Asian Pacific Journal of Tropical Disease* · December 2015

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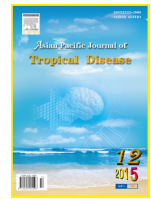


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## Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Review article

doi: 10.1016/S2222-1808(15)60959-8

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A systematic review of the effects of Iranian pharmaceutical plant extracts on *Giardia lamblia*Hajar Ziaei Hezarjaribi<sup>1</sup>, Taher Elmi<sup>2</sup>, Mohammad Saaïd Dayer<sup>3</sup>, Shirzad Gholami<sup>4</sup>, Mahdi Fakhar<sup>1</sup>, Mostafa Akbariqomi<sup>5</sup>, Fatemeh Ghaffarifar<sup>3\*</sup><sup>1</sup>Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran<sup>2</sup>Department of Parasitology and Mycology, Iran University of Medical Sciences, Tehran, Iran<sup>3</sup>Department of Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran<sup>4</sup>Toxoplasmosis Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran<sup>5</sup>Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

## ARTICLE INFO

## Article history:

Received 8 Sep 2015

Received in revised form 17 Nov 2015

Accepted 4 Dec 2015

Available online 8 Dec 2015

## Keywords:

*Giardia lamblia*

Iranian pharmaceutical plants

Aqueous extract

Chloroformic extract

Hydroalcoholic extract

## ABSTRACT

This study aimed to provide a systematic review regarding anti-*Giardia* effect of different Iranian plant extracts used *in vivo* and *in vitro* on cysts and trophozoites. Many reports indicated that most of plant extracts used as anti-*Giardia* were obtained from Liliaceae, Apiaceae, Asteraceae, and Myrtaceae. These extracts included different fractions such as aqueous, alcoholic and chloroform extracts as well as Soxhlet extraction of juice or essence. The findings of this review showed that hydroalcoholic extract of asafoetida, *Chenopodium botrys*, and chloroformic extract of feverfew (*Tanacetum parthenium*) have the maximum effect (100% efficacy) on *in vitro* application against *Giardia*. However, the highest *in vivo* effect of 100% therapeutic significance was recorded for the extract of *Allium sativum* at 80 mg/mL concentration. Given the plant species richness of Iran in terms of herbal medicines with fewer side effects, it can be a good alternative to chemical drugs used to treat giardiasis.

## 1. Introduction

Giardiasis is one of the most common diseases in many parts of the world including Iran. The disease is caused by a protozoan *Giardia* transmitted via fecal-oral routes in water and food. Swallowing 10 cysts is enough to be contagious with *Giardia lamblia* (*G. lamblia*) [1,2]. Annually, 280 million human infections are estimated to occur worldwide [3]. Although symptoms of giardiasis are not alike in different patients, indigestion, bloating, mild to severe diarrhea and malabsorption are the common symptoms. Some people may experience acute symptoms such as cramping, abdominal pain, bloating, belching, anorexia, epigastric tenderness, nausea and sometimes vomiting, weight loss, general weakness and diarrhea. Patients may also show increased fat excretion in their stools [4,5].

## 2. Treatment of giardiasis

Recently, prescribed treatment of giardiasis includes various chemical agents such as metronidazole, tinidazole, ornidazole, mebendazole and albendazole. These drugs have many side effects; besides, their impact is not always certain and some have been reported to induce carcinogenic and mutagenic complications in animal models. Also some of these drugs are contraindicated during pregnancy [6-8]. Therefore, the use of these medications is controversial because of the reported side effects and drug resistance [9]. To reduce the adverse effects of chemical drugs, researchers are now looking for different ways to find safe alternative remedies including the use of herbs and natural foods as proposed by World Health Organization [10].

\*Corresponding author: Fatemeh Ghaffarifar, Parasitology and Entomology Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, P. O. 14115-331, Iran.

Tel: +982182884553

Fax: +982182884555

E-mail: [ghaffarifar@modares.ac.ir](mailto:ghaffarifar@modares.ac.ir)

Foundation Project: Supported by Hajar Ziaei Hezarjaribi grant (Grant No. 2106).

2.1. Effect of medicinal plant extracts on protozoan *G. lamblia*

Medicinal herbs are the whole or part of plants that contain

effective substances which constitute less than 1% dry weight and are of medicinal properties[11]. Researchers have recorded significant effects of various medicinal plant extracts on many parasites such as *Giardia*. Giardiasis is one of the most common parasitic infections of early ages that can cause growth retardation in children[12]. Some of medicinal plants reported as effective drugs with fewer side effects against giardiasis are as follows.

#### 2.1.1. *Carum copticum* (*C. copticum*)

The plant *C. copticum* belongs to Apiaceae family. The essential oils and compounds such as thymol, terpinene and fliandren are important ingredients present mainly in seeds which are also the richest part of oil in the plant. *Carum* extracts have been demonstrated to have analgesic, antinausea, antifatulent, and antiparasitic effects[13]. Several studies showed that the plant was effective against Gram-positive and Gram-negative bacteria and prevents the growth of several fungi[14]. A study on anti-giardiasis effects of this plant revealed that the plant extract has the ability to destroy all *Giardia* cysts *in vitro* at a concentration of 100 mg/mL[13].

#### 2.1.2. *Tanacetum parthenium* (*T. parthenium*)

*T. parthenium* belongs to the family Asteraceae, a bunch of flowering plants, which produce yellow, white and sometimes purple flowers. The plant prefers more rough and sandy soil and grows near the edge of crop fields. This herb is one of the most effective drugs for the treatment of allergic asthma[11].

*T. parthenium* extract has been successfully used in the treatment of abdominal pain, flatulence, nervous headaches, migraine, colitis and nerve compression[15]. The flowers contain a large amount of terpene lactones as well as parthenolide of which antiparasitic effects have been documented[11]. *In vitro* studies showed that crude extracts of *T. parthenium* inhibited the growth of epimastigote of *Trypanosoma cruzi*[16]. In another study, chloroform extract of *T. parthenium* was showed to be more effective on *Giardia* cyst than hydroalcoholic extract[11]. The plant extract was suggested to be an herbal treatment for giardiasis in Balb/c mice[8].

#### 2.1.3. *Zataria multiflora* (*Z. multiflora*)

This plant is a member of Lamiaceae family of which antibacterial, antiparasitic and antifungal actions have been proven in various studies[17,18]. Thymol and carvacrol are the main antibacterial constituents of essential oil of the plant[17]. A recent study has demonstrated the effect of *Z. multiflora* extract on the growth of *Staphylococcus aureus*[18]. Keramati *et al.* reported that extracts of leaves and flowering shoots of the plant were effective in treating dimethylbenzanthracene induced carcinoma in prostate of the desert mouse[19]. Farsangi found that *Z. multiflora* extract caused higher mortality of *G. lamblia* cysts (91.1%) than metronidazole (89%), and was recommended as a suitable alternative drug for giardiasis[17].

#### 2.1.4. *Sambucus ebulus* (*S. ebulus*)

*S. ebulus* belongs to the plant family Caprifoliaceae and its elderberry stems, leaves, roots and fruits are used in the treatment of various diseases[20]. A study showed anti-inflammatory effects of *S. ebulus* on inflammation caused by burns, eczema and edema[21]. Antiparasitic and antibacterial effects of elderberry have also been documented[22]. In an *in vitro* study, Rahimi-Esboei *et al.* have shown that the plant extract has good effect on giardiasis[20].

#### 2.1.5. *Asafoetida*

*Ferula asafoetida* is an important medicinal plant of the family Apiaceae. Resin obtained from this plant is used in traditional medicine to treat many diseases particularly parasitic diseases[23]. There is evidence on carminative and analgesic effects of the plant extracts. Kiasalari *et al.* reported that alcoholic extract of this resinous plant was an effective herbal medicine in the treatment of epilepsy and seizures in mice[24]. Its aqueous extracts also showed *in vitro* anti-*Trichomonas* activity[25]. Studies were undertaken to examine the effect of aqueous and alcoholic extracts of this plant on *Giardia* cysts, and the results showed that the alcoholic extract was more effective than the aqueous extract in destroying *Giardia* cysts in 5 h[23].

#### 2.1.6. *Artemisia annua* (*A. annua*)

*A. annua* is a flowering plant from the family Asteraceae. The plant contains artemisinin, a sesquiterpene lactone[26]. This chemical has an internal peroxide bridge which appears to be indispensable for the chemotherapeutic activity, hence it was used as an antimalarial drug in the tropics[27]. *In vitro* studies have shown that artemisinin can kill parasites and bacteria and reduce human malaria mortality by 50% as compared to standard anti-malaria drug quinoline[11]. This herb has, also, anti-cancer properties and is of selective toxicity to breast cancer cells[28]. Hydroalcoholic and chloroform extracts of this herb indicated anti-giardiasis activity *in vitro* at concentration of 100 mg/mL[26].

#### 2.1.7. *Eucalyptus globules* (*E. globules*)

Being a member of the Myrtaceae family, this plant contains cineole as an active ingredient in its leaves. Cineole of *Eucalyptus* has anti-cancer, anti-inflammatory and anti-pain properties[29]. Several studies showed that *Eucalyptus* extract has, also, anti-fungal and anti-malarial activities[30]. Some *in vitro* studies has proved the effectiveness of *Eucalyptus* against giardiasis[29].

#### 2.1.8. *Heracleum glabrescens* (*H. glabrescens*) and *Satureja hortensis* (*S. hortensis*)

*H. glabrescens* is a plant from family Apiaceae (Umbelliferae) and *S. hortensis* belongs to Lamiaceae (Labiatae). The mint *H. glabrescens* contains resin and anethole and therefore has antibacterial and antiparasitic activities. Also, leaf extract of *S. hortensis* herb includes thymol and carvacrol in its composition which are of antibacterial properties[31]. Safarnejad *et al.* showed that alcoholic

extracts of *S. hortensis* and *H. glabrescens* caused 84.3% and 44% mortality of *Giardia* cysts, respectively[29].

### 2.1.9. *Chenopodium botrys* (*C. botrys*)

Belonging to the family Amaranthaceae, *C. botrys* was used as antiparasitic drug in traditional medicine. Ziaei *et al.* provided evidence on cytotoxic effect of aqueous extract of *C. botrys* on *Trichomonas vaginalis* trophozoite at concentrations equal to 0.1% and 0.01% in 1 to 4 h[32]. However, examining fruit and seed extracts of *C. botrys*, Rezaeemanesh *et al.* showed that the extracts have anti-*Giardia* activity[33].

### 2.1.10. *Allium*

The most important plants in the genus *Allium* in the family Liliaceae are garlic [*Allium sativum* (*A. sativum*)], onion [*Allium cepa*, *Allium paradoxum* (*A. paradoxum*)], shallots (*Allium ascalonicum*)[34]. It has been reported that the administration of sulfoxide amino acids found in garlic and onion improved the diabetic conditions such as glucose tolerance, weight loss and depletion of liver glycogen. In other studies, oral administration of garlic extract increased gastric acid and pepsin hypersecretion in animals and humans[11,35].

Azadbakht *et al.* found the liquid extracts of onion, garlic, and shallot to be effective *in vitro* on *Giardia* cysts[34]. The highest mortality of *Giardia* cysts was recorded for all extracts at 24 °C after 3 h incubation[33]. *In vivo* studies revealed that ethanolic extract of *A. paradoxum* was more effective on *Giardia* cysts than the chloroform extract at a concentration equal to 100 mg/mL[11]. Elmi has also shown that ethanolic extract of *A. paradoxum* was significantly more effective than the chloroform extract in the treatment of giardiasis[36]. However, chloroform extract of garlic was of better performance in the treatment of mice infected with *Giardia* parasite[36].

Potential teratogenic side effects of metronidazole and other drugs used to treat giardiasis make it necessary to find alternative drugs; hence identifying antiparasitic compounds from other sources, such as herbs, seems essential. Therefore, the present study was intended to review the anti-giardiasis effects of various medicinal plants studied in Iran.

### 2.2. Effect of various plant extracts on *Giardia* cysts *in vitro*

Most studies concerning the effect of different plant extracts on *Giardia* parasite were carried out under *in vitro* conditions in Iran. And 10 out of 26 studies used alcoholic fractions for their *in vitro* analyses. Evaluating the results of these studies illustrated that only alcoholic and chloroform extracts were able to destroy *Giardia* cysts. In the alcoholic extract, both asafetida and *C. botrys* at a concentration of 20 mg/mL were the most effective to produce 100% killing effect on *G. lamblia* cysts. However, chloroform extracts of *A. sativum* and *T. parthenium* at concentrations equal to 8 mg/mL and 100 mg/mL respectively were able to produce 100% mortality on *Giardia* cysts (Table 1).

Among the extracts studied *in vitro*, the boiled extract of *Z.*

*multiflora* has the lowest effect (7%) in destroying cysts (Table 1).

**Table 1**

Effects of plant extracts on *G. lamblia* cysts under *in vitro* condition in Iran.

Scientific name	Fractions	EC (mg/mL)	Time (h)	Drug effects (%)	Organs	Ref.
<i>C. copticum</i>	Aqueous	700	3	10.0	Seed	[13]
	Hydroalcoholic	75	1	94.1	Seed	[13]
	Essence	6	1	95.5	Seed	[13]
<i>T. parthenium</i>	Hydroalcoholic	100	3	87.0	Flower	[11]
	Chloroform	100	3	100.0	Flower	[11]
<i>Z. multiflora</i>	Essence	-	1	91.1	Dried plant	[17]
	Boiling	-	1	7.0	Dried plant	[17]
	Soxhlet	-	1	13.8	Dried plant	[17]
<i>S. ebulus</i>	Alcoholic	100	1	78.0	Fruit	[20]
Asafoetida	Aqueous	20	5	57.2	Gum	[23]
	Alcoholic	20	4	100.0	Gum	[23]
<i>A. annua</i>	Hydroalcoholic	100	3	94.0	Stem, leaf	[26]
<i>E. globules</i>	Alcoholic	200	1	63.3	Leaf	[29]
<i>H. glabrescens</i>	Alcoholic	200	1	44.0	Seed	[29]
<i>S. hortensis</i>	Alcoholic	200	1	84.3	Leaf	[29]
<i>C. botrys</i>	Aqueous	20	5	66.1	Seed	[32]
	Alcoholic	20	5	100.0	Seed	[32]
<i>A. sativum</i>	Liquid	-	3	43.2	Bulb	[33]
	Chloroform	8	8	100.0	Bulb	[37]
<i>Allium cepa</i>	Liquid	-	3	40.8	Bulb	[33]
	AA	-	3	33.6	Bulb	[33]
<i>A. paradoxum</i>	Hydroalcoholic	100	3	97.0	Leaf	[11]
	Chloroform	100	3	96.0	Leaf	[11]
Vinegar	Juice	1	3	40.6	Fruit	[38]
Lemon	Juice	1	3	28.3	Fruit	[38]
Vinifera	Juice	1	3	16.2	Fruit	[38]

AA: *Allium ascalonicum*; EC: Effective concentration.

### 2.3. Study on the effects of different plant extracts on *Giardia* cysts *in vivo*

Because of the difficulties associated with *in vivo* methods, less studies have been undertaken to examine the effects of plant extracts on *G. lamblia* in animal or human. Major obstacles of *in vivo* study are as follows: 1) infecting animals; 2) providing appropriate conditions for animals; 3) keeping the experimental animal alive until the end of the study. Nonetheless, some authors have studied the effects of medicinal plants extracts on *G. lamblia* parasite *in vivo*[8,36-39].

Safar Harandi *et al.* indicated that extract of *A. sativum* was the most effective in treating *Giardia* infection in mice at 80 mg/mL in 3 days[37]. Others showed that the extracts of *T. parthenium* and *A. paradoxum* had 92% and 88% therapeutic effect on mice infected with *G. lamblia*, showing acceptable results (Table 2)[8,36].

Hydroalcoholic extract of *A. paradoxum* was of the lowest efficacy (71%) among the *in vivo* applied plant extracts (Table 2).

**Table 2**

Effects of plant extracts on *G. lamblia* cysts under *in vivo* condition in Iran.

Scientific name	Fractions	EC (mg/mL)	Time (day)	DE (%)	Organs	Ref.
<i>A. paradoxum</i>	Hydroalcoholic	100	3	71	Leaf, bulb	[36]
	Chloroform	100	3	88	Bulb	[36]
<i>T. parthenium</i>	Chloroform	100	3	92	Flower	[8]
<i>A. sativum</i>	Chloroform	80	3	100	Bulb	[37]

EC: Effective concentration; DE: Drug effects.

### 3. Discussion

*G. lamblia* is one of the most common intestinal parasites in many parts of the world as well as Iran, particularly in the northern areas

of Iran[8]. Giardiasis infection varies in different regions of Iran from 1% to 25%. It has been estimated that about 280 million *Giardia* infections occur worldwide in humans annually[3]. Chemical treatment of the disease by metronidazole, furazolidone, and tinidazole drugs has many side effects including carcinogenic implications in women and children. Yet, their efficacy is unknown and their use is contraindicated during pregnancy[2,8].

In recent years, reports on parasite resistance to the above drugs have been in rise[9]. Different methods have been suggested to eliminate *Giardia* cyst and trophozoite. One of such methods suggested by World Health Organization is the use of herbs and natural foods[10]. In recent years, the use of medicinal herbs has increased due to their low side effects.

Based on *in vivo* and *in vitro* studies, extracts of medicinal plants such as garlic, *C. copticum*, asafoetida, *T. parthenium*, and *A. paradoxum* showed promising effects in the elimination of *Giardia* cysts particularly when used in combination as anti-*Giardia* complex drug[8,23,33,36].

The diversity of plants used in different studies have shown interesting results. Being member of different plant families, each herb showed a specified antiparasitic effect. For example, *A. annua* (Asteraceae) has shown more anti-giardiasis effect in comparison with *E. globules* (Myrtaceae)[26,29]. Also, *A. sativum* extract (Amaryllidaceae) was more effective than *T. parthenium* extract (Asteraceae) in treating mice infected with *G. lamblia* as revealed by Safar Harandi *et al.*[37] and Elmi *et al.*[8]. However, differences between human and murine strains of *Giardia* is an important factor which may affect the results.

It can be drawn from the published studies that in addition to the plant species, the kind of chemical fractions (ethanolic, aqueous, chloroform) and experimental environments (*in vitro* and *in vivo*) play determinant role in the outcomes. For example, the hydroalcoholic extracts showed greater anti-*Giardia* effects compared with aqueous extracts[13]. Also, *T. parthenium* extract showed a greater anti-*Giardia* effect when applied *in vitro* than *in vivo*[8,11]. This is because under *in vivo* conditions micro-organisms would be affected by both herbal drugs and animal immune system simultaneously. Consequently, the results would be more realistic under *in vivo* conditions. We may recommended that the plant extracts showing promising antiparasitic effect in test tube should be examined under *in vivo* conditions for therapeutic significance (where possible).

Nowadays, the anti-bacteria properties of various plants such as *Artemisia aucheri*, *Thymus vulgaris*, *Salvia officinalis*, *E. globules*, *Matricaria recutita*, and *Zingiber* against *Pseudomonas*, *Brucella*, acute bronchitis, inflammation, diarrhea, and seizures have been proven[39-41]. Due to the side effects of chemical drugs, more comprehensive studies should be undertaken to examine the medical properties of other native plants of this country. Researches should be also directed towards different plant fractions. Since various parts of plants including roots, leaves, stems, and fruits

have different antibacterial constituents, it is recommended to ensure the examination of all parts of the medicinal plants. Also, significant *in vitro* results should be fortified with more *in vivo* experiments to pave the way for introduction of more plant-based drugs for a better health and safer life.

## Conflict of interest statement

We declare that we have no conflicts of interest.

## Acknowledgments

This study is funded by Hajar Ziaei Hezarjaribi grant with Grant No. 2106. The authors wish to thank all colleagues in Parasitology Departments of Tarbiat Modares University and Molecular and Cell Biology Research Center of Mazandaran University of Medical Sciences.

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